

? b 155

16may03 08:12:33 User208669 Session D2289.1

\$0.31 0.089 DialUnits File

\$0.01 TELNET

\$0.32 Estimated cost this search

\$0.32 Estimated total session cost 0.089 DialUnits

File 155:MEDLINE(R) 1966-2003/May W2

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\*File 155: Medline has been reloaded and accession numbers have changed. Please see HELP NEWS 155.

Set Items Description

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? s au=lebkowski

S1 0 AU=LEBKOWSKI

? s au=lebkowski?

S2 120 AU=LEBKOWSKI?

? s py=1988

S3 365037 PY=1988

? s s2 and s3

120 S2

365037 S3

S4 4 S2 AND S3

? t s4/7/1-4

4/7/1

DIALOG(R)File 155:MEDLINE(R)

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06572961 90198200 PMID: 3273199

Optimizing electroporation parameters for a variety of human hematopoietic cell lines.

McNally M A; Lebkowski J S; Okarma T B; Lerch L B

Div. of Molecular Biology, Applied ImmuneSciences, Inc., Menlo Park, CA

94025-1109.

BioTechniques (UNITED STATES) Oct 1988, 6 (9) p882-6, ISSN

0736-6205 Journal Code: 8306785

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The parameters affecting electroporation of four human hematopoietic cell lines were investigated. The optimal conditions for electroporation are described for both transient and stable expression of foreign genes. A correlation exists between the levels of transient gene expression and stable transfection frequency. In addition, linear DNA yields higher stable transfection frequencies than supercoiled DNA. The cumulative results

indicate that electroporation is a simple and useful method for obtaining transient and stable expression of foreign genes in human hematopoietic cells.

Record Date Created: 19900510

Record Date Completed: 19900510

4/7/2

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

06246659 89262497 PMID: 3249520

[Intracranial epidural implantation of a Gaeltec ICT/b sensor in the light of our experience]

Srodezaszkowa nadwarcowkowa implantacja czujnika Gaeltec ICT/b w swietle doswiadczen własnych.

Lebkowski W; Kozlowski A; Kollataj J

Kliniki Neurochirurgii AM w Białymostku.

Neurologia i neurochirurgia polska (POLAND) Sep-Oct 1988, 22 (5)

p476-8, ISSN 0028-3843 Journal Code: 0101265

Document type: Journal Article

Languages: POLISH

Main Citation Owner: NLM

Record type: Completed

Record Date Created: 19890710

Record Date Completed: 19890710

4/7/3

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

06122835 89138181 PMID: 2465249

Platelet aggregability in patients with common carotid artery ligation.

Marak Z; Kłoczko J; Lewko J; Wojtukiewicz M; Bielawiec M; Lebkowski J

Department of Neurosurgery, Medical School, Białystok, Poland.

Folia haematologica - internationales Magazin für klinische und

morphologische Blutforschung (GERMANY, EAST) 1988, 115 (5) p689-93,

ISSN 0015-556X Journal Code: 0374615

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Increased platelet aggregability is regarded as being a sensitive indicator of the initiation of thrombotic processes. Platelet aggregation was analysed in blood taken from the common carotid artery before and 30 min after its ligation in 3 patients, as well as in the venous blood of 14 patients in the late postoperative period. No tendency towards increasing platelet aggregation was observed in either of the groups investigated.

Record Date Created: 19890329

Record Date Completed: 19890329

4/7/4

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

06025033 89039822 PMID: 2847025

Adeno-associated virus: a vector system for efficient introduction and integration of DNA into a variety of mammalian cell types.

Lebkowski J S; McNally M M; Okarma T B; Lerch L B

Applied ImmuneSciences, Inc., Division of Molecular Biology, Menlo Park, California 94025.

Molecular and cellular biology (UNITED STATES) Oct 1988, 8 (10) p3988-96, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Adeno-associated virus (AAV) is a single-stranded DNA parvovirus that is dependent on adenovirus or herpesvirus for reproductive functions. We describe the construction of recombinant AAV vectors containing the chloramphenicol acetyltransferase gene or the neomycin phosphotransferase gene. These vectors carried their respective genes into a wide variety of cell types, including primary skin fibroblasts and hematopoietic cells.

Infection efficiencies varied with cell type and ranged up to 3.0%.

Coinfection of two different recombinant viruses was also used to introduce two different sequences simultaneously into a given cell. Finally, methods for obtaining recombinant AAV vectors with minimal contamination of wild-type virus are described. These various attributes of AAV vectors make them a viable DNA transduction system.

Record Date Created: 19881221

Record Date Completed: 19881221

? log hold

16may03 08:14:53 User208669 Session D2289.2

\$1.43 0.445 DialUnits File155

\$0.84 4 Type(s) in Format 7

\$0.84 4 Types

\$2.27 Estimated cost File155

\$0.70 TELNET

\$2.97 Estimated cost this search

\$3.29 Estimated total session cost 0.535 DialUnits

Logoff: level 02.14.01 D 08:14:53

? b 155

16may03 09:48:39 User208669 Session D2290.1

\$0 26 0.076 DialUnits File1

\$0 26 Estimated cost File1

\$0 26 Estimated total session cost 0.076 DialUnits

File 155:MEDLINE(R) 1966-2003/May W2

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\*File 155: Medline has been reloaded and accession numbers have changed. Please see HELP NEWS 155.

Set Items Description

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? ds

Set Items Description

S1 1760 AAV OR ADENOASSOCAT? OR ADENO(W)ASSOCAT?

S2 931056 DT=REVIEW?

S3 247 S1 AND S2

S4 97 S1/T1 AND S3

S5 45 LIMIT? AND S3

S6 97530 PROMOTER?

S7 8 S6 AND (S4 OR S5)

?rs7771-8

7/7/1

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

14731987 22302501 PMID: 12413426

Gene delivery to the eye using adeno-associated viral vectors.

Martin Keith R G; Klein Ronald L; Quigley Harry A

Wilmer Eye Institute, Wilmer 122, Johns Hopkins Hospital, 600 North Wolfe

Street, Baltimore, MD 21287, USA.

Methods (San Diego, Calif.) (United States) Oct 2002, 28 (2) p267-75

, ISSN 1046-2023 Journal Code: 9426302

Contract/Grant No.: EY 01765; EY; NEI; EY 02120; EY; NEI

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Adeno-associated virus (AAV) vectors provide a useful way to deliver genes to the eye. They have a number of important properties which make them suitable for this purpose, not least their lack of significant pathogenicity and the potential for long-term transfection of retinal cells. The optimal methods for AAV-mediated gene delivery are determined by the location and characteristics of the target cell type. Efficient gene delivery to photoreceptors and pigment epithelial cells following subretinal injection of AAV has been achieved in various animal models.

AAV-mediated gene therapy has been shown to slow photoreceptor loss in rodent models of primary photoreceptor diseases and in dogs with a naturally occurring disease similar to human Leber's congenital amaurosis (LCA). Efficient gene delivery to other cell types such as retinal ganglion cells (RGCs), however, has been more problematic. In this article, we review the potential uses of AAV-mediated gene delivery to the eye. We describe the selection of an appropriate AAV vector for ocular gene transfer studies and discuss the techniques used to deliver the virus to the eye and to assess ocular transfection. We emphasize our techniques for successful gene transfer to retinal ganglion cells, which have often proven challenging to transfet with high efficiency. Using a modified AAV incorporating a chicken beta-actin (CBA) promoter and the woodchuck hepatitis posttranscriptional regulatory element, we describe how our techniques allow approximately 85% of rat retinal ganglion cells to be transfected within 2 weeks of a single intravitreal virus injection. Our techniques facilitate the study of the pathogenesis of RGC diseases such as glaucoma and the development of novel new treatments based on gene therapy. Copyright 2002 Elsevier Science (USA) (40 Refs.)

Record Date Created: 20021104

Record Date Completed: 20030423

7/7/2

DIALOG(R)File 155:MEDLINE(R)

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14731982 22302496 PMID: 12413421

Promoters and regulatory elements that improve adeno-associated virus transgene expression in the brain.

Fitzsimons Helen L; Bland Ross J; During Matthew J

CNS Gene Therapy Center, Department of Neurosurgery, Thomas Jefferson

University, Philadelphia, PA 19107, USA.

Methods (San Diego, Calif.) (United States) Oct 2002, 28 (2) p227-36

, ISSN 1046-2023 Journal Code: 9426302

Document type: Journal Article; Review; Review Literature

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Since the first demonstration of central nervous system (CNS) transduction with recombinant adeno-associated virus, improvements in vector production and promoter strength have lead to dramatic increases in the number of cells transduced and the level of expression within each cell. The improvements in promoter strength have resulted from a move away from the original cytomegalovirus (CMV) promoter toward the use of hybrid CMV-based promoters and constitutive cellular promoters. This review summarizes and compares different promoters and regulatory elements that have been used with rAAV as a reference toward achieving a high level of rAAV-mediated transgene expression in the CNS. Copyright 2002 Elsevier Science (USA) (69 Refs.)

Record Date Created: 20021104  
 Record Date Completed: 20030423

7/7/3  
 DIALOG(R)File 155: MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.  
 14731981 22302495 PMID: 12413420

Regulation of gene expression in adeno-associated virus vectors in the brain.  
 Haberman Rebecca P; McCown Thomas J  
 Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. rahabs@med.unc.edu  
*Methods* (San Diego, Calif.) (United States) Oct 2002, 28 (2) p219-26  
 , ISSN 1046-2023 Journal Code: 9426302  
 Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM  
 Record type: Completed  
 Regulated adeno-associated virus (AAV) vectors have broad utility in both experimental and applied gene therapy, and to date, several regulation systems have exhibited a capability to control gene expression from viral vectors over two orders of magnitude. The tetracycline responsive system has been the most used in AAV, although other regulation systems such as RU486- and rapamycin-responsive systems are reasonable options. AAV vectors influence how regulation systems function by several mechanisms, leading to increased background gene expression and restricted induction. Methods to reduce background expression continue to be explored and systems not yet tried in AAV may prove quite functional. Although regulated promoters are often assumed to exhibit ubiquitous expression, the tropism of different neuronal subtypes can be altered dramatically by changing promoters in recombinant AAV vectors. Differences in promoter-directed tropism have significant consequences for proper expression of gene products as well as the utility of dual vector regulation. Thus regulated vector systems must be carefully optimized for each application. Copyright 2002 Elsevier Science (USA) (45 Refs.)

Record Date Created: 20021104

Record Date Completed: 20030423  
 7/7/4  
 DIALOG(R)File 155: MEDLINE(R)  
 (c) format only 2003 The Dialog Corp. All rts. reserv.  
 11983844 99428941 PMID: 10499153  
 Gene transfer and models of gene therapy for the myocardium.  
 Alexander M Y; Webster K A; McDonald P H; Prentice H M  
 Division of Molecular Genetics, Institute of Biomedical and Life Sciences, University of Glasgow, UK.  
*Clinical and experimental pharmacology & physiology* (AUSTRALIA) Sep

1999, 26 (9) p661-8, ISSN 0305-1870 Journal Code: 0425076  
 Document type: Journal Article; Review; Review; Literature  
 Languages: ENGLISH

Main Citation Owner: NLM  
 Record type: Completed

1. Gene transfer into the myocardium can be achieved through direct injection of plasmid DNA or through the delivery of viral vectors, either directly or through the coronary vasculature. Direct DNA injection has proven extremely valuable in studies aimed at characterizing the activities of promoter elements in cardiac tissue and for examining the influence of the pathophysiological state of the myocardium on expression of transferred foreign genes. 2. Viral vectors, in particular adenoviruses and adeno-associated virus, are capable of transfecting genetic material with high transduction efficiencies and have been applied to a range of model systems for *in vivo* gene transfer. Efficient gene transfer has been achieved into the coronary vessels and surrounding myocardium by intracoronary infusion of adenovirus. 3. Because the immunogenicity of viral vectors can limit transgene expression, much attention has been paid to strategies for circumventing this, including the development of new modified adenovirus and adeno-associated virus vectors that do not elicit significant inflammatory responses. While cellular transplantation may prove valuable for the repair of myocardial tissue, confirmation of its value awaits establishment of a functional improvement in the myocardium following cell grafting. 4. Because gene transfer into the myocardium can now be achieved with high efficiency in the absence of significant inflammatory responses, the ability to regulate foreign gene expression in response to an endogenous disease phenotype will enable the development of new effective viral vectors with direct clinical applicability for specified therapeutic targets. (85 Refs.)

Record Date Created: 19991026

Record Date Completed: 19991026  
 7/7/5  
 DIALOG(R)File 155: MEDLINE(R)  
 (c) format only 2003 The Dialog Corp. All rts. reserv.  
 11180457 98056844 PMID: 9395731  
 Gene therapy for the hemophilias.  
 Walter J; High K A.

Department of Cardiothoracic Surgery, University of Vienna, Austria.  
*Advances in veterinary medicine* (UNITED STATES) 1997, 40 p119-34,  
 ISSN 1093-975X Journal Code: 9714525  
 Document type: Journal Article; Review; Review, Academic  
 Languages: ENGLISH  
 Main Citation Owner: NLM  
 Record type: Completed  
 There are many lines of evidence that suggest the eventual success of gene therapy as a treatment strategy for hemophilia. Because current

treatment protocols using plasma-derived or recombinant proteins are far from ideal, the safe and efficient substitution of the defective gene by a normal copy of the gene, or at least its addition, would be of great benefit to the patient and may even be a potential cure. However, the construction of efficient gene therapy vehicles has proven quite difficult in the past and, so far, there is no system that promises to have all the desired features without any serious disadvantages. In general, either the levels of transgene expression are too low (because of the low titers achieved during the generation of the virus) or shortlived (e.g., because of the specific shut-off of the transferred promoter) as is often seen with retroviruses, or in the case of adenoviral vectors, expression is limited because of a strong immune response of the host. Clearly, much work remains to be done to optimize these promising though still imperfect vector systems. In the case of adenovirus, the development of less immunogenic vectors or in vivo modulation of the host immune system may hold promise for improvements. Reports by Yang et al. (1995) and Kay et al. (1995) are promising steps in the direction of immunomodulation. Both attenuate the immune reaction to the adenoviral vector by simultaneous application of either an interleukin or an immunoglobulin, respectively. When IL-2 was administered, the amounts of IgA were reduced and successful administration of a second dose of virus was possible. When CTLA4-Ig, an immunoglobulin that blocks the second signal during antigen presentation, was administered, a markedly prolonged expression of the transgene resulted. In vivo trials with AAV vectors have been carried out for some diseases (Flotte et al., 1993; Kaplitt et al., 1994) but not for hemophilia.

Advances in high-titer AAV vector preparation will make this approach more feasible. The pace continues to quicken in the development of nonviral modes of gene delivery (Perales et al., 1994). Although these results are encouraging for the future of gene therapy as a treatment for genetic diseases, much work remains to be done to make this potential alternative a reality for treatment of hemophilia. (48 Refs.)

Record Date Created: 19980109

Record Date Completed: 19980109

7/7/6  
DIALOG(R)File 155: MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.  
09727976 21525173 PMID: 11667959

Size does matter: overcoming the adeno-associated virus packaging limit.

Flotte T R  
Powell Gene Therapy Center, University of Florida, Gainesville, Florida  
32610-0266, USA. flottt@pedr.ufl.edu  
Respiratory research (England) 2000, 1 (1) p16-8, ISSN 1465-9921  
Journal Code: 101090633

Document type: Journal Article; Review, Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Recombinant adeno-associated virus (rAAV) vectors mediate long-term gene transfer without any known toxicity. The primary limitation of rAAV has been the small size of the virion (20 nm), which only permits the packaging of 4.7 kilobases (kb) of exogenous DNA, including the promoter, the polyadenylation signal and any other enhancer elements that might be desired. Two recent reports (D Duan et al: Nat Med 2000, 6:595-598; Z Yan et al: Proc Natl Acad Sci USA 2000, 97:6716-6721) have exploited a unique feature of rAAV genomes, their ability to link together in doublets or strings, to bypass this size limitation. This technology could improve the chances for successful gene therapy of diseases like cystic fibrosis or Duchenne muscular dystrophy that lead to significant pulmonary morbidity.

(17 Refs.)

Record Date Created: 200111022

Record Date Completed: 200111205

7/7/7

DIALOG(R)File 155: MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.  
09399592 21165820 PMID: 11269333

Gene therapy for hemophilia.

Chuah M K; Collen D; VandenDriessche T  
Center for Transgene Technology and Gene Therapy, Flanders  
Interuniversity Institute for Biotechnology, University of Leuven, Belgium.  
Journal of gene medicine (England) Jan-Feb 2001, 3 (1) p3-20, ISSN  
1099-498X Journal Code: 9815764

Document type: Journal Article; Review, Review, Academic

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Hemophilia A and B are X-chromosome linked recessive bleeding disorders that result from a deficiency in factor VIII (FVIII) and factor IX (FIX) respectively. Though factor substitution therapy has greatly improved the lives of hemophiliac patients, there are still limitations to the current treatment that have triggered interest in alternative treatments by gene therapy. Significant progress has recently been made in the development of gene therapy for the treatment of hemophilia A and B. These advances parallel the technical improvements of existing vector systems including MoMLV-based retroviral, adenoviral and AAV vectors, and the development of new delivery methods such as lentiviral vectors, helper-dependent adenoviral vectors and improved non-viral gene delivery methods.

Therapeutic and physiologic levels of FVIII and FIX could be achieved in FVIII- and FIX-deficient mice and hemophiliac dogs by different gene therapy approaches. Long-term correction of the bleeding disorders and in some cases a permanent cure has been realized in these preclinical studies. However, the induction of neutralizing antibodies often precludes stable phenotypic correction. Another complication is that certain promoters are

prone to transcriptional inactivation in vivo, precluding long-term FVIII or FIX expression. Several gene therapy phase I clinical trials are currently ongoing in patients suffering from severe hemophilia A or B. No significant adverse side-effects were reported, and semen samples were negative for vector sequences by sensitive PCR assays. Most importantly, some subjects report fewer bleeding episodes and occasionally have very low levels of clotting factor activity detected. The results from the extensive preclinical studies in normal and hemophilic animal models and encouraging preliminary clinical data indicate that the simultaneous development of different strategies is likely to bring a permanent cure for hemophilia one step closer to reality. (180 Refs.)

Record Date Created: 20010327  
 Record Date Completed: 20010517

7/7/8  
 DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.  
 09046843 20341995 PMID: 10880823  
 Adeno-associated virus vectors: activity and applications in the CNS.

Peel A.L., Klein R.L.

Buck Center for Research in Aging, POB 638, 8001 Redwood Blvd., Novato, CA 94948, USA.apeel@buckcenter.org

Journal of neuroscience methods (NETHERLANDS) Jun 1 2000, 98 (2)  
 p95-104, ISSN 0165-0270 Journal Code: 7905558  
 Document type: Journal Article; Review; Review, Tutorial  
 Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Transgenic strategies are useful for functional studies and they may also lead to novel therapies. Controlling transgene expression in defined cell populations over time is increasingly important for both functional and gene therapy experiments. The adeno-associated virus (AAV) vector may provide sufficient spatio-temporal control of gene expression for these purposes. This paper reviews in vivo somatic gene transfer methodology using AAV. Advantageous features of this system include neuronal gene expression that is: (1) efficient; (2) long-lived; and (3) non-toxic. Thus, AAV-mediated gene transfer is a good method for functional genomic research. From characterizing vector activity in the brain using different combinations of promoters and transgenes in the mid to late 1990s, researchers continue to discover novel uses of AAV for both basic and clinical neuroscience. (66 Refs.)

Record Date Created: 20000824  
 Record Date Completed: 20000824

? log hold

16may03 09:56:11 User208669 Session D2290.2

\$4.66 1.457 DialUnits File155  
 \$0.00 150 Type(s) in Format 6

\$1.68 8 Type(s) in Format 7

\$1.68 158 Types

\$6.34 Estimated cost File155

\$1.86 TELNET

\$8.20 Estimated cost this search

\$8.46 Estimated total session cost 1.533 DialUnits Logoff: level 02.14.01 D 09:56:11

? b 155

16may03 10:43:08 User208669 Session D2291.1

\$0.28 0.081 DialUnits File1

\$0.01 TELNET

\$0.29 Estimated cost this search

0.081 DialUnits 0.29 Estimated total session cost

7/7/8  
 DIALOG(R)File 155:MEDLINE(R) 1966-2003May W2

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 \*File 155: Medline has been reloaded and accession numbers have changed. Please see HELP NEWS 155.

Set Items Description

? ds

Set	Items	Description
S1	1760	AAV OR ADENOASSOCIAT? OR ADENOW(W)ASSOCIAT?
S2	1663	MINI?(3N)PROMOTER
S3	0	S2 AND S3
S4	4	S2 AND S1
S5	803422	SPECIFIC
S6	391	S1 AND S3
S7	97530	PROMOTER?
S8	126	S6 AND S7
S9	27297	EXPRESS?(3N)S5
S10	25	S9 AND S8
S11	1210293	REDUC? OR MINIMAL OR MINIMIZE OR DELET?
S12	97381	PROMOTER OR PROMOTERS
S13	84	S1 AND S11 AND S12
S14	11	S1 AND S11(3N)S12
S15	2	SMALL?(3N)S12 AND S1

? t s4/7/1-4

4/7/1  
 DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.  
 10127813 22103650 PMID: 12084814

Adeno-associated viral vector-mediated hypoxia response element-regulated gene expression in mouse ischemic heart model.

Su Hua; Arakawa-Hoyt Janice; Kan Yuet Wai  
 Cardiovascular Research Institute, University of California, 513  
 Parnassus Avenue, Room U432, San Francisco, CA 94143-0793, USA.

Proceedings of the National Academy of Sciences of the United States of America (United States) Jul 9 2002, 99 (14) p9480-5, ISSN 0027-8424  
 Journal Code: 7505876

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Intramyocardial injection of genes encoding angiogenic factors could provide a useful approach for the treatment of ischemic heart disease. However, uncontrolled expression of angiogenic factors *in vivo* may cause some unwanted side effects, such as hemangioma formation, retinopathy, and arthritis. It may also induce occult tumor growth and atherosclerotic plaque progression. Because hypoxia-inducible factor 1 is up-regulated in a variety of hypoxic conditions and it regulates gene expression by binding to a cis-acting hypoxia-responsive element (HRE), we propose to use HRE, found in the 3' end of the erythropoietin gene to control gene expression in ischemic myocardium. A concatemer of nine copies of the consensus sequence of HRE isolated from the erythropoietin enhancer was used to mediate hypoxia induction. We constructed two adeno-associated viral vectors in which LacZ and vascular endothelial growth factor (VEGF) expressions were controlled by this HRE concatemer and a minimal simian virus 40 promoter. Both LacZ and VEGF expression were induced by hypoxia and/or anoxia in several cell lines transduced with these vectors. The

functions of these vectors in ischemic myocardium were tested by injecting them into normal and ischemic mouse myocardium created by occlusion of the left anterior descending coronary artery. The expression of LacZ gene was induced eight times and of VEGF 20 times in ischemic myocardium compared with normal myocardium after the viral vector transduction. Hence, HRE is a good candidate for the control of angiogenic factor gene expression in ischemic myocardium.

Record Date Created: 20020710  
 Record Date Completed: 20020808

4/7/2  
 DIALOG(R)File 155.MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.  
 10068509 22013996 PMID: 11889137

Cardiomyocyte-specific gene expression following recombinant adeno-associated viral vector transduction.

Aikawa Ryuchi; Huggins Gordon S; Snyder Richard O  
 Cardiovascular Biology Laboratory, Harvard School of Public Health,  
 Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115,  
 USA.

p18979-85, ISSN 0021-9258 Journal Code: 2985121R  
 Contract/Grant No.: R01 HL54592-06; HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Recombinant adeno-associated viral (rAAV) vectors hold promise for delivering genes for heart diseases, but cardiac-specific expression by the use of rAAV has not been demonstrated. To achieve this goal rAAV vectors were generated expressing marker or potentially therapeutic genes under the control of the cardiac muscle-specific alpha myosin heavy chain (MHC) gene promoter. The rAAV-MHC vectors expressed in primary cardiomyocytes with similar kinetics to rAAV-CMV; however, expression by the rAAV-MHC vectors was restricted to cardiomyocytes. rAAV vectors have low cytotoxicity, and it is demonstrated here that rAAV fails to induce apoptosis in cardiomyocytes compared with a recombinant adenoviral vector. rAAV-MHC or rAAV-CMV vectors were administered to mice to determine the specificity of expression *in vivo*. The rAAV-MHC vectors expressed specifically in cardiomyocytes, whereas the control rAAV-CMV vector expressed in heart, skeletal muscle, and brain. rAAV-MHC transduction resulted in long term (16 weeks) expression of human growth hormone following intracardiac, yet not intramuscular, injection. Finally, we defined the minimal MHC enhancer/promoter sequences required for specific and robust *in vivo* expression in the context of a rAAV vector. For the first time we describe a panel of rAAV vectors capable of long term cardiac specific expression of intracellular and secreted proteins.

Record Date Created: 20020520  
 Record Date Completed: 20020624

4/7/3

DIALOG(R)File 155.MEDLINE(R)

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09112860 20411448 PMID: 10954575

Novel transcriptional regulatory signals in the adeno-associated virus terminal repeat A/D junction element.

Haberman R P; McCown T J; Samulski R J  
 UNC Gene Therapy Center, University of North Carolina, Chapel Hill, North Carolina 27599, USA.

Journal of virology (UNITED STATES) Sep 2000, 74 (18) p8732-9,  
 ISSN 0022-538X Journal Code: 0113724  
 Contract/Grant No.: DK51880; DK; NIDDK; NS35633; NS; NINDS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed  
 Adeno-associated virus (AAV) type 2 vectors transfer stable, long-term gene expression to diverse cell types *in vivo*. Many gene therapy

Journal of biological chemistry (United States) May 24 2002, 277 (21)

applications require the control of long-term transgene expression, and AAV vectors, similar to other gene transfer systems, are being evaluated for delivery of regulated gene expression cassettes. Previously, we (R. P. Haberman, T. J. McCown, and R. J. Samulski, *Gene Ther.* 5:1604-1611, 1998) demonstrated the use of the tetracycline-responsive system for long-term regulated expression in rat brains. In that study, we also observed residual expression in the "off" state both *in vitro* and *in vivo*, suggesting that the human cytomegalovirus (CMV) major immediate-early minimal promoter or other *cis*-acting elements (AAV terminal repeats [TR]) were contributing to this activity. In the present study, we identify that the AAV TR, minus the tetracycline-responsive minimal CMV promoter, will initiate mRNA expression from vector templates. Using deletion analysis and specific PCR-derived TR reporter gene templates, we mapped this activity to a 37-nucleotide stretch in the A/T elements of the TR. Although the mRNA derived from the TR is generated from a non-TATA box element, the use of mutant templates failed to identify function of canonical initiator sequences as previously described. Finally, we demonstrated the presence of green fluorescent protein expression both *in vitro* and *in vivo* in brain by using recombinant virus carrying only the TR element. Since the AAV terminal repeat is a necessary component of all recombinant AAV vectors, this TR transcriptional activity may interfere with all regulated expression cassettes and may be a problem in the development of novel TR split gene vectors currently being considered for genes too large to be packaged.

Record Date Created: 20000927

Record Date Completed: 19931101

?t s107/4 6-12

DIALOG(R)File 155.MEDLINE(R)  
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107/4  
Neuron-specific transduction in the rat septohippocampal or nigrostriatal

pathway by recombinant adeno-associated virus vectors.  
Klein R L; Meyer E M; Peel A L; Zolotukhin S; Meyers C; Muzychka N; King

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Experimental neurology (UNITED STATES) Apr 1998, 150 (2) p183-94,

ISSN 0014-4886 Journal Code: 0370712

Contract/Grant No.: AG00196; AG; NIA; GM 35723; GM; NIGMS; PPG AG10485;

AG; NIA; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Viral vector-mediated gene transfer in brain can provide a means for gene therapy and functional studies. However, robust and persistent transgene expression in specific populations of the adult brain has been difficult to achieve. In an attempt to produce localized and persistent transduction in rat brain, we compared recombinant adeno-associated virus (rAAV) vectors incorporating either the immediate early cytomegalovirus (CMV) promoter or the neuron-specific enolase (NSE) promoter. Transduction in hippocampus resulting from the NSE promoter-containing construct was more efficient and persistent than that resulting from the CMV promoter-containing construct. Most hippocampal cells transduced with the NSE promoter had multipolar neuron morphology. Neurons with glutamatergic morphology were transduced weakly. In order to produce a local supply of neurotrophic factor to cells that degenerate under certain disease and experimental conditions, the NSE promoter was utilized to drive expression of brain-derived neurotrophic factor (BDNF) in medial septum or substantia nigra. In this construct, the NSE promoter drives dicistronic expression of BDNF and an enhanced version of green fluorescent protein (GFP). We estimated 3000-15,000 GFP-positive cells per injection of rAAV into septum or substantia nigra, a transduction ratio of 5-20 infectious virus particles per transduced cell. This

can greatly enhance the level of transcription initiation at initiator elements. A binding site for the YY1 transcription factor, located at the initiation site of the adeno-associated virus P5 promoter, functions as an initiator element; a synergistic enhancement of its activity is observed *in vitro* when upstream Sp1 binding sites are present. Here we report that this synergistic activation probably occurs through protein-protein interactions.

Record Date Created: 19931101

Record Date Completed: 19931101

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DIALOG(R)File 155.MEDLINE(R)  
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107/4  
Neuron-specific transduction in the rat septohippocampal or nigrostriatal

pathway by recombinant adeno-associated virus vectors.  
Klein R L; Meyer E M; Peel A L; Zolotukhin S; Meyers C; Muzychka N; King

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ISSN 0014-4886 Journal Code: 0370712

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A basal level of transcription is usually observed when all but a small region of DNA has been deleted from a eukaryotic gene promoter. These promoter elements, which are necessary and sufficient for specific transcription initiation, are referred to as minimal or core promoter elements. One element that is commonly present in a core promoter is the initiator. It has been demonstrated that the presence of Sp1 binding sites

frequency may be sufficient for trophic factor gene therapy as well as for investigating specific protein function in "topical (i.e., localized) transgenic" animals produced by rAAV. Copyright 1998 Academic Press.

Record Date Created: 19980522

Record Date Completed: 19980522

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DIALOG(R)File 155: MEDLINE(R)

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11192761 98069311 PMID: 9406237

Targeting gene therapy to cancer: a review.

Dachs G U; Dougherty G J; Stratford I J; Chaplin D J

Gray Laboratory, Mount Vernon Hospital, Northwood, UK.  
dachs@graylab.ac.uk

Oncology research (UNITED STATES) 1997, 9 (6-7) p313-25, ISSN 0965-0407 Journal Code: 9208097

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In recent years the idea of using gene therapy as a modality in the treatment of diseases other than genetically inherited, monogenic disorders has taken root. This is particularly obvious in the field of oncology where currently more than 100 clinical trials have been approved worldwide. This report will summarize some of the exciting progress that has recently been made with respect to both targeting the delivery of potentially therapeutic genes to tumor sites and regulating their expression within the tumor microenvironment. In order to specifically target malignant cells while at the same time sparing normal tissue, cancer gene therapy will need to combine highly selective gene delivery with highly specific gene expression, specific gene product activity, and, possibly, specific drug activation. Although the efficient delivery of DNA to tumor sites remains a formidable task, progress has been made in recent years using both viral (retrovirus, adenovirus, adeno-associated virus) and nonviral (liposomes, gene gun, injection) methods. In this report emphasis will be placed on targeted rather than high-efficiency delivery, although those would need to be combined in the future for effective therapy. To date delivery has been targeted to tumor-specific and tissue-specific antigens, such as epithelial growth factor receptor, c-kit receptor, and folate receptor, and these will be described in some detail. To increase specificity and safety of gene therapy further, the expression of the therapeutic gene needs to be tightly controlled within the target tissue. Targeted gene expression has been analyzed using tissue-specific promoters (breast-, prostate-, and melanoma-specific promoters) and disease-specific promoters (carcinoembryonic antigen, HER-2/neu, Myc-Max response elements, DF3/MUC). Alternatively, expression could be regulated externally with the use of radiation-induced promoters or tetracycline-responsive elements. Another

novel possibility that will be discussed is the regulation of therapeutic gene products by tumor-specific gene splicing. Gene expression could also be targeted at conditions specific to the tumor microenvironment, such as glucose deprivation and hypoxia. We have concentrated on hypoxia-targeted gene expression and this report will discuss our progress in detail.

Chronic hypoxia occurs in tissue that is more than 100-200 microns away from a functional blood supply. In solid tumors hypoxia is widespread both because cancer cells are more prolific than the invading endothelial cells that make up the blood vessels and because the newly formed blood supply is disorganized. Measurements of oxygen partial pressure in patients' tumors showed a high percentage of severe hypoxia readings (less than 2.5 mmHg), readings not seen in normal tissue. This is a major problem in the treatment of cancer, because hypoxic cells are resistant to radiotherapy and often to chemotherapy. However, severe hypoxia is also a physiological condition specific to tumors, which makes it a potentially exploitable target. We have utilized hypoxia response elements (HRE) derived from the oxygen-regulated phosphoglycerate kinase gene to control gene expression in human tumor cells in vitro and in experimental tumors. The list of genes that have been considered for use in the treatment of cancer is extensive. It includes cytokines and costimulatory cell surface molecules intended to induce an effective systemic immune response against tumor antigens that would not otherwise develop. Other inventive strategies include the use of internally expressed antibodies to target oncogenic proteins (intrabodies) and the use of antisense technology (antisense oligonucleotides, antigenes, and ribozymes). This report will concentrate more on novel genes encoding produg activating enzymes, so-called suicide genes (Herpes simplex virus thymidine kinase, Escherichia coli nitroreductase, E. (ABSTRACT TRUNCATED) (105 Refs.)

Record Date Created: 19980112

Record Date Completed: 19980112

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DIALOG(R)File 155: MEDLINE(R)

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11178024 98054331 PMID: 9391123

Tissue-specific expression of herpes simplex virus thymidine kinase gene delivered by adeno-associated virus inhibits the growth of human hepatocellular carcinoma in athymic mice.

Su H; Lu R; Chang J C; Kan Y W

Department of Laboratory Medicine, University of California, Third and Parnassus Avenues, Room U426, San Francisco, CA 94143-0724, USA. Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 9 1997, 94 (25) p13891-6, ISSN 0027-8424 Journal Code: 7505876

Contract/Grant No.: DK16666; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

About 70% of hepatocellular carcinomas are known to express alpha-fetoprotein, which is normally expressed in fetal but not in adult livers. To induce herpes simplex virus-thymidine kinase expression in these cancer cells, we constructed an adeno-associated viral vector containing the HSV-TK gene under the control of the alpha-fetoprotein enhancer and albumin promoter. We previously demonstrated in vitro that although this vector can transduce a variety of human cells, only transduced AFP and albumin-expressing hepatocellular carcinoma cell lines were sensitive to killing by ganciclovir (GCV). In the present study, we explored the effect of this vector on hepatocellular carcinoma cells in vivo. Subcutaneous tumors generated in nude mice by implanting hepatocellular carcinoma cells previously transduced with this vector shrank dramatically after treatment with GCV. Bystander effect was also observed on the tumors generated by mixing transduced and untransduced cells. To test whether the tumor cells can be transduced by the virus *in vivo*, we injected the recombinant adeno-associated virus into tumors generated by untransduced hepatocarcinoma cell line. Tumor growth were retarded after treatment with GCV. These experiments demonstrate the feasibility of *in vivo* transduction of tumor cell with rAAV.

Record Date Created: 19980115  
 Record Date Completed: 19980115

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DIALOG(R)File 155:MEDLINE(R)  
 (c) format only 2003 The Dialog Corp. All rts. reserv.  
 10984968 97338119 PMID: 9192666  
 Efficient photoreceptor-targeted gene expression *in vivo* by recombinant adeno-associated virus.

Flannery J G; Zolotukhin S; Vaquero M I; LaVail M M; Muzyczka N;  
 Hauswirth W W  
 School of Optometry and Neuroscience Group, University of California, Berkeley, CA 94720, USA.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Jun 24 1997, 94 (13) p6916-21, ISSN 0027-8424  
 Journal Code: 7505876  
 Contract/Grant No.: EY07864; EY; NEI; EY11123; EY; NEI; GM53723; GM; NIGMS; +  
 Document type: Journal Article  
 Languages: ENGLISH  
 Main Citation Owner: NLM  
 Record type: Completed

We describe a general approach for achieving efficient and cell type-specific expression of exogenous genes in photoreceptor cells of the mammalian retina. Recombinant adeno-associated virus (rAAV) vectors were used to transfer the bacterial lacZ gene or a synthetic green fluorescent

protein gene (gfp) to mouse or rat retinas after injection into the subretinal space. Using a proximal murine rod opsin promoter (+86 to -385) to drive expression, reporter gene product was found exclusively in photoreceptors, not in any other retinal cell type or in the adjacent retinal pigment epithelium. GFP-expressing photoreceptors typically encompassed 10-20% of the total retinal area after a single 2-microl injection. Photoreceptors were transduced with nearly 100% efficiency in the region directly surrounding the injection site. We estimate approximately 2.5 million photoreceptors were transduced as a result of the single subretinal inoculation. This level of gene transfer and expression suggests the feasibility of genetic therapy for retinal disease. The gfp-containing rAAV stock was substantially free of both adenovirus and wild-type AAV, as judged by plaque assay and infectious center assay, respectively. Thus, highly purified, helper virus-free rAAV vectors can achieve high-frequency tissue-specific transduction of terminally differentiated, postmitotic photoreceptor cells.

Record Date Created: 19970721  
 Record Date Completed: 19970721

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DIALOG(R)File 155:MEDLINE(R)  
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 10793853 97083335 PMID: 8929909  
 Autonomous parvovirus transduction of a gene under control of tissue-specific or inducible promoters.

Maxwell I H; Spitzer A L; Long C J; Maxwell F  
 University of Colorado Cancer Center and Health Sciences Center, Denver 80262, USA.

Gene therapy (ENGLAND) Jan 1996, 3 (1) p28-36, ISSN 0969-7128  
 Journal Code: 9421525  
 Contract/Grant No.: CA-50285; CA; NCI  
 Document type: Journal Article  
 Languages: ENGLISH  
 Main Citation Owner: NLM

Record type: Completed

Several classes of viruses are in use, or are being developed, as gene therapy vectors. Viruses with small genomes containing few essential genes have the advantage of requiring only simple complementation systems to allow packaging of foreign DNA, substituted for the entire viral coding sequences. Retroviruses and the dependent parvovirus AAV (adeno-associated virus) have been used in this way, and both possess an efficient integration mechanism which should allow long-term expression of transduced genes. In some situations, however, long-term persistence may be undesirable and there is a need for small, non-integrating viral vectors. Autonomous parvoviruses, such as Lulli, have potential as such vectors for short-term expression of therapeutic genes. We previously described recombinants of Lulli that transduced reporter genes, expressed using the

viral constitutive promoter, P4. We have now generated several recombinants containing regulated promoters. A virus including a liver-specific enhancer directed 10- to 20-fold preferential expression of the luciferase reporter in transduced human hepatoma (HepG2) versus HeLa cells. In additional LullI recombinants, the luciferase reporter was linked with chimeric promoters containing binding sequences for either the yeast GAL4 protein or the bacterial tetracycline repressor. Luciferase expression was strongly activated when these viruses were used to infect cells containing a cognate trans-activator (GAL4 or tTA, a tetracycline repressor fusion with VP16 of herpes simplex), introduced by transfection. The response to tTA could be abolished, or reduced in a graded manner, by exposure of the infected cells to tetracycline. Further results suggested that an increase in basal expression, apparently mediated by the viral left terminal inverted repeat, could be minimized by interposing polyadenylation signals between this sequence and the promoter. These results confirm that appropriate transcriptional regulation can be achieved for genes transduced by an autonomous parvovirus vector. Such vectors therefore show promise for the delivery of therapeutic genes in situations requiring cell-specific, short-term expression, eg in targeting suicide genes for ablation of cancer cells.

Record Date Created: 19970328

Record Date Completed: 19970328

10/7/10

DIALOG(R)File 155: MEDLINE(R)

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10733853 97083335 PMID: 8929909

Autonomous parvovirus transduction of a gene under control of tissue-specific or inducible promoters.

Maxwell I H; Spitzer A L; Long C J; Maxwell F

University of Colorado Cancer Center and Health Sciences Center, Denver

80262, USA.

Gene therapy (ENGLAND) Jan 1996, 3 (1) p28-36, ISSN 0969-7128

Journal Code: 9421525

Contract/Grant No.: CA-50285; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Several classes of viruses are in use, or are being developed, as gene therapy vectors. Viruses with small genomes containing few essential genes have the advantage of requiring only simple complementation systems to allow packaging of foreign DNA, substituted for the entire viral coding sequences. Retroviruses and the dependent parvovirus AAV (adeno-associated virus) have been used in this way, and both possess an efficient integration mechanism which should allow long-term expression of transduced genes. In some situations, however, long-term persistence may be

undesirable and there is a need for small, non-integrating viral vectors. Autonomous parvoviruses, such as LullI, have potential as such vectors for short-term expression of therapeutic genes. We previously described recombinants of LullI that transduced reporter genes, expressed using the viral constitutive promoter, P4. We have now generated several recombinants containing regulated promoters. A virus including a liver-specific enhancer directed 10- to 20-fold preferential expression of the luciferase reporter in transduced human hepatoma (HepG2) versus HeLa cells. In additional LullI recombinants, the luciferase reporter was linked with chimeric promoters containing binding sequences for either the yeast GAL4 protein or the bacterial tetracycline repressor. Luciferase expression was strongly activated when these viruses were used to infect cells containing a cognate trans-activator (GAL4 or tTA, a tetracycline repressor fusion with VP16 of herpes simplex), introduced by transfection. The response to tTA could be abolished, or reduced in a graded manner, by exposure of the infected cells to tetracycline. Further results suggested that an increase in basal expression, apparently mediated by the viral left terminal inverted repeat, could be minimized by interposing polyadenylation signals between this sequence and the promoter. These results confirm that appropriate transcriptional regulation can be achieved for genes transduced by an autonomous parvovirus vector. Such vectors therefore show promise for the delivery of therapeutic genes in situations requiring cell-specific, short-term expression, eg in targeting suicide genes for ablation of cancer cells.

Record Date Created: 19970328

Record Date Completed: 19970328

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DIALOG(R)File 155: MEDLINE(R)

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10445492 96252193 PMID: 8646533

Adeno-associated virus 2-mediated transduction and erythroid cell-specific expression of a human beta-globin gene.

Zhou S Z; Li Q; Stamatoyannopoulos G; Srivastava A

Department of Medicine, Indiana University School of Medicine, Indianapolis 46202-5120, USA.

Gene therapy (ENGLAND) Mar 1996, 3 (3) p223-9, ISSN 0969-7128

Journal Code: 9421525

Contract/Grant No.: AI-26323; AI; NIAID; HL-48342; HL; NHLBI; HL-53586;

HL; NHLBI; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Recombinant adeno-associated virus 2 (AAV) virions were constructed that contained the genomic copy of a normal human beta-globin gene marked with a 4-bp Clai linker, and the herpesvirus thymidine kinase (TK) promoter-driven

bacterial gene for resistance to neomycin (*v* beta m-globin), as well as those containing the DNase I-hypersensitive site 2 (HS-2) from the locus control region (LCR) of the human beta-globin gene cluster (vHS2-beta m-globin). These recombinant virions were used to infect a human erythroleukemia cell line which normally does not express the beta-globin gene (K562), or a human nasopharyngeal carcinoma cell line (KB). Cell populations resistant to G418, a neomycin analogue, were obtained following infections with the recombinant virions, indicating high-efficiency transduction of the chimeric gene as well as functional activity of the transduced neo gene in both cell types. Southern blot analysis using a human beta-globin DNA probe substantiated stable integration of the exogenous beta-globin allele in these cells. There was no expression of the transduced beta-globin gene in K562 or KB cells infected with the v beta m-globin virus. High-level expression of the transduced beta-globin gene occurred only in the vHS2-beta m-globin virus-infected K562 cells, but not in KB cells, as determined by Northern blot as well as RNase protection analyses. Expression of the human beta-globin protein could also be detected in approximately 10-20% of the vHS2-beta m-globin virus-infected K562 cells. These studies suggest that the AAV-based vector system may prove useful for high-efficiency globin gene transfer in human hematopoietic cells.

Record Date Created: 19960725  
Record Date Completed: 19960725

10/7/12

DIALOG(R)File 155:MEDLINE(R)

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10441112 96247788 PMID: 8699328  
Synthesis of human globin polypeptides mediated by recombinant adeno-associated virus vectors.

Ohi S, Kim B C

Center for Sickle Cell Disease, College of Medicine, Howard University, Washington, D.C. 20059, USA.

Journal of pharmaceutical sciences (UNITED STATES) Mar 1996, 85 (3) p274-81, ISSN 0022-3549 Journal Code: 2985195R  
Contract/Grant No.: K14 HL01989; HL; NHLBI

Document type: Journal Article  
Languages: ENGLISH

Main Citation Owner: NLM  
Record type: Completed

Adeno-associated virus, serotype 2 (AAV2)-based chimeric plasmids that harbored a near-full-length human alpha- or beta-globin cDNA were constructed. The cDNAs were spliced into an AAV plasmid, pAAV delta K, downstream from the viral P40 promoter, substituting the capsid gene region. The correctness of the insertion with regard to the transcription polarity was ascertained by both restriction enzyme analysis and DNA sequencing. One of the constructs, pAAVchBBLCR, contained the

erythroid-specific enhancer elements, the locus control region, HS1 and HS2, to ensure an efficient and tissue-specific gene expression. Use of a defective complementing helper, pAVXB (Dixit, M.; et al. Gene 1991, 104, 253-257.) and adenovirus 2 made it possible to prepare recombinant AAVs (rAAVs). Infection of human 293 cells (embryonal kidney cell line) with the resultant rAAV (AAVchBB) and cotransfection of mouse erythroleukemia (MEL) cells with the beta-globin construct (pAAVchBBLCR) and an alpha-globin construct (pAAVchHAB) triggered efficient synthesis of human globin polypeptides in the cells, as analyzed by biochemical and immunohistochemical means. The LCR made the construct respond to an inducer, N,N-hexamethylenebisacetamide, the amount of expressed human beta-globin reaching a similar level as the endogenous mouse beta-globin in MEL cells. Electroporation of mouse bone marrow hematopoietic stem/progenitor cells with the constructs dramatically increased the number of benzidine-positive cells in liquid suspension culture, indicating expression and synthesis of a human hemoglobin in these cells. Thus, the rAAV constructs may be useful for gene therapy of hemoglobinopathies.

Record Date Created: 19960905  
Record Date Completed: 19960905  
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DIALOG(R)File 155:MEDLINE(R)

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11490227 98374322 PMID: 9707617

Efficient expression of CFTR function with adeno-associated virus vectors that carry shortened CFTR genes.

Zhang L, Wang D, Fischer H, Fan P D; Widdicombe J H; Kan Y W; Dong J Y  
Gene Therapy Core Center for Cystic Fibrosis and Genetic Diseases and  
Department of Laboratory Medicine, University of California, San Francisco,  
CA 94143, USA.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Aug 18 1998, 95 (17) p10158-63, ISSN 0027-8424 Journal Code: 7505876

Contract/Grant No.: DK/HL46177; DK; NIDDK; DK47766; DK; NIDDK  
Document type: Journal Article  
Languages: ENGLISH

Main Citation Owner: NLM  
Record type: Completed

Adeno-associated virus (AAV)-based vectors have been shown to be effective in transferring the cystic fibrosis gene (CFTR) into airway epithelial cells in animal models and in patients. However, the level of CFTR gene expression has been low because the vector cannot accommodate the CFTR gene together with a promoter. In this study, we described a strategy to reduce the size of the CFTR cDNA to allow the incorporation of an effective promoter with the CFTR gene into AAV vectors. We engineered and tested 20 CFTR mini-genes containing deletions that were targeted to regions that may contain nonessential sequences. Functional analyses showed

that four of the shortened CFTRs (one with combined deletions) retained the function and the characteristics of a wild-type CFTR, as measured by open probability, time voltage dependence, and regulation by cAMP. By using an AAV vector with a P5 promoter, we transduced these short forms of CFTR genes into target cells and demonstrated high levels of CFTR expression. We also demonstrated that smaller AAV/CFTR vectors with a P5 promoter expressed the CFTR gene more efficiently than larger vectors or a vector in which CFTR gene was expressed from the AAV inverted terminal repeat sequence. The CFTR mini-gene with combined deletions was packaged into AAV virions more efficiently, generated higher titers of transducing virions, and more effectively transferred CFTR function into target cells. These new vectors should circumvent the limitations of AAV vector for CFTR expression. Our strategy also may be applicable to other genes, the sizes of which exceed the packaging limit of an AAV vector.

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S1 1354 AAV OR ADENOASSOCIAT? OR ADENO(W)ASSOCIAT?

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S4 11 S2 AND S1

S5 51918 SPECIFIC

S6 351 S1 AND S5

S7 26728 PROMOTER?

S8 173 S6 AND S7

S9 3668 EXPRESS?(3N)S5

S10 52 S9 AND S8  
S11 52378 REDUC? OR MINIMAL OR MINIMIZE OR DELET?  
S12 26650 PROMOTER OR PROMOTERS  
S13 158 S1 AND S11 AND S12  
S14 13 S1 AND S11(3N)S12  
S15 6 SMALL?(3N)S12 AND S1  
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0244505 DBR Accession No.: 1999-12652

Control of erythropoietin delivery by doxycycline in mice after intramuscular injection of adeno-associated vector - potential use in beta-thalassemia gene therapy

AUTHOR: Bohl D; Salvetti A; Moullier P; +Heard J M

CORPORATE SOURCE: Laboratoire Retrovirus et Transfert Genetique, Institut Pasteur, 28 rue du Dr. Roux, 75724, Paris, France.

email:jmheard@pasteur.fr  
JOURNAL: Blood (92, 5, 1512-17) 1998  
ISSN: 0006-4971 CODEN: BLOOA W

LANGUAGE: English

ABSTRACT: Vector rAAV-ET contains 2 transcriptional units oriented in opposite directions, with a central bidirectional SV40 virus polyadenylation site. Sequences encoding the chimeric transcription factor rtTA, which confers doxycycline-inducible expression, were inserted downstream of a retro virus long terminal repeat promoter. A minimal human cytomegalovirus promoter flanked with tetracycline operator motifs, to which the rtTA protein could bind, controlled the transcription of mouse erythropoietin (Epo) cDNA. Thus, expression of the murine Epo cDNA was modulated in response to doxycycline. The vector was injected i.m. in normal mice. Hematocrit and serum Epo concentrations were modulated over a 29-wk period in response to the presence or absence of doxycycline in the drinking water of these animals. There was no evidence of an immune response directed against the rtTA protein. The systemic delivery of potentially very high amounts of Epo and the possibility of preventing polycythemia by a tight control of gene expression by doxycycline may allow consideration of rAAV-mediated gene transfer into muscles for future human trials for gene therapy of beta-thalassemia. (35 ref)

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DIALOG(R)File 357:Derwent Biotech Res.  
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0210739 DBR Accession No.: 97-05860 PATENT  
New collision constructs - novel collision construct containing 1st and 2nd promoter and a reporter gene and vector expression in a host cell for

transcription activation inhibitor identification

AUTHOR: Giese K; Escobedo J

CORPORATE SOURCE: Emeryville, CA, USA.

PATENT ASSIGNEE: Chiron 1997

PATENT NUMBER: WO 9710360 PATENT DATE: 970320 WPI ACCESION NO.: 97-202254 (9718)

PRIORITY APPLIC. NO.: US 689926 APPLIC. DATE: 960815

NATIONAL APPLIC. NO.: WO 96US13845 APPLIC. DATE: 960821

LANGUAGE: English

**ABSTRACT:** A collision construct (1) is new and contains a DNA molecule harboring a 1st regulatory sequence containing a 1st minimal promoter;

a reporter gene (alkaline phosphatase (EC-3.1.3.1), luciferase, chloramphenicol-acetyltransferase (EC-3.2.1.28), beta-galactosidase, (EC-3.2.1.23), beta-glucuronidase (EC-3.2.1.31) and green fluorescent protein) under the control of the 1st promoter, and a 2nd regulatory sequence containing a 2nd promoter. Also claimed are: a vector containing (1) and a nucleotide sequence allowing for the expression of (1) in a eukaryotic or prokaryotic host cell; a host cell (e.g. mammal (HeLa), insect, yeast or bird cell); and a kit containing the above.

The 1st and 2nd promoters are selected from promoters derived from a virus (retro, vaccinia, herpes, hepatitis, papilloma, adeno or adeno-associated virus), phage, prokaryotic or eukaryotic gene. The 2nd promoter is derived from a cytomegalo, herpes simplex, hepatitis or HIV virus. The products can be used to identify inhibitors of promoter or transcription activation. Such inhibitors may turn off genes responsible for e.g. cancer and viral infections. (56pp)

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0208125 DBR Accession No.: 97-03246 PATENT

Recombinant adeno virus with virus gene under control of inducible promoter

- adeno virus and adeno-associated virus gene therapy vector construction

AUTHOR: Latta M; Orsini C; Perricaudet M; Prost E; Vigne E; Yeh P

CORPORATE SOURCE: Antony, France.

PATENT ASSIGNEE: Rhone-Poulenc-Rorer 1997

PATENT NUMBER: WO 9700947 PATENT DATE: 970109 WPI ACCESION NO.: 97-087374 (9708)

PRIORITY APPLIC. NO.: FR 957570 APPLIC. DATE: 950623

NATIONAL APPLIC. NO.: WO 96FR968 APPLIC. DATE: 960620

LANGUAGE: French

**ABSTRACT:** A recombinant adeno virus (AV) is claimed in which expression of at least one homologous or heterologous virus gene is controlled by an inducible promoter (IP). Also new are: (1) use of the AV containing at least one adeno-associated virus (AAV) gene under the control of a tetracycline-IP (preferably Op2/Tk) for preparation of AAV; (2) a 293 cell having integrated into its genome an expression DNA cassette for a

transcription activator containing a protein able to bind to the regulatory sequence of an IP present in the adeno virus, plus a second protein that activates transcription; and (3) promoter Op2/Tk (specified DNA sequence and protein sequence). The IP contains a minimal promoter and a regulatory sequence (specified 67 and 75 bp DNA sequences) containing at least one copy of the tetracycline operator. The new method for making AA V is simple and provides higher viral titers than standard methods. AV can be produced safely since they do not contain replicative particles. AV and AA V are used as gene therapy vectors to deliver a wide range of therapeutic genes, especially for the treatment of cancer, restenosis and other proliferative diseases. (63pp)

? ts107/28 32-36 43 45-48  
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DIALOG(R)File 357:Derwent Biotech Res.

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0235956 DBR Accession No.: 99-06057 PATENT

Use of modified insulin-like growth factor-I- recombinant somatomedin-C with muscle-specific promoter, useful for improving muscle strength and mass and for promoting glucose clearance from muscle tissue

AUTHOR: Sweeney HL; Rosenthal NA

CORPORATE SOURCE: Philadelphia, PA, USA; Charlestown, MA, USA.

PATENT ASSIGNEE: Univ.Pennsylvania; Gen.Hosp.Charlestown 1999

PATENT NUMBER: WO 9910013 PATENT DATE: 990304 WPI ACCESION NO.: 99-190469 (9916)

PRIORITY APPLIC. NO.: US 57201 APPLIC. DATE: 970825

NATIONAL APPLIC. NO.: WO 98US17428 APPLIC. DATE: 980825

LANGUAGE: English

**ABSTRACT:** A DNA sequence (1) encoding somatomedin-C (SC) or a modified or active portion of SC is claimed. Also claimed are: (1) flanked by a

SV40 virus intron at the 5' end and by an SV40 virus polyadenylation signal sequence at the 3' end, the coding region being operably linked to a muscle-specific promoter such as skeletal alpha-actin promoter, a muscle-specific troponin promoter; a composition comprising the viral vector, preferably an adeno-associated virus vector, encoding (1); a host cell comprising (1) or the vector; a kit for increasing muscle mass and strength comprising (1); and a non-human vertebrate transgenic animal comprising (1). The products are used for: preserving or enhancing muscle mass in aging humans; healing injured muscle more efficiently/rapidly; controlling muscle mass during disease and/or during prolonged stays in reduced gravity; cosmetic body sculpting; and promoting glucose clearance from diabetic muscle tissue. Recombinant expression of SC in muscle fibers may activate satellite cells. This technique does not have the deleterious side-effects of steroids for stimulating muscle hypertrophy. (46pp)

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DIALOG(R)File 357:Derwent Biotech Res.

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0226914 DBR Accession No.: 98-08511 PATENT  
Expression of polynucleotides in mammals - recombinant adeno-associated virus vector construction and use in gene therapy

AUTHOR: Snyder R; Danos O; Cohen L; Kay M; Thompson A R  
CORPORATE SOURCE: Foster City, CA, USA; Seattle, WA, USA.

PATENT ASSIGNEE: Somatix-Ther.; Univ.Washington-Seattle 1998  
PATENT NUMBER: WO 9824479 PATENT DATE: 980611 WPI ACCESION NO.: 98-333055 (9829)

PRIORITY APPLIC. NO.: US 882044 APPLIC. DATE: 970625  
NATIONAL APPLIC. NO.: WO 97US21398 APPLIC. DATE: 971202

LANGUAGE: English

**ABSTRACT:** A new method of expressing a polynucleotide (I) in a mammal involves administering virus particles containing a recombinant adeno-associated virus (AAV) vector containing (I) to liver cells of the mammal. The method can be used to treat a liver disease in a mammal, where (I) encodes a therapeutic protein. (I) may be linked to a liver tissue-specific promoter. Also claimed is a method of determining the presence of wild-type AAV and infectious AAV, generated by recombination of helper AAV and a vector AAV containing a transgene in a sample of recombinant AAV, using DNA amplification. The vector AAV contains nucleotide sequences or has an order of nucleotide sequences different from that of wild-type AAV. The methods permit expression of diffusible proteins in the liver which provides access to the circulation and permits systemic delivery of therapeutic proteins. The proteins can be used for treatment of blood disease e.g. hemophilia, metabolic disease e.g. familial hypercholesterolemia, liver-specific disease e.g. glycogen storage disease, or liver tumors. (63pp)

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DIALOG(R)File 357:Derwent Biotech Res.

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0223528 DBR Accession No.: 98-05125

Viral sequences enable efficient and tissue-specific expression of transgenes in Xenopus - adeno-associated virus inverted terminal repeat sequence effect on tissue-specific gene expression

AUTHOR: Fu Y; Wang Y; +Evans S M

CORPORATE AFFILIATE: Univ.California  
CORPORATE SOURCE: Department of Medicine, University of California, San Diego, La Jolla, CA 92093-0613, USA. email:syevans@ucsd.edu

JOURNAL: Nat.Biotechnol. (16, 3, 253-57) 1998  
ISSN: 1087-0156 CODEN: NABIF

LANGUAGE: English

**ABSTRACT:** A novel strategy was developed for efficient and stable expression of transgenes driven by both ubiquitous and tissue-specific promoters, involving direct DNA injection into developing Xenopus

laevis embryos. The process approaches the efficiency of restriction endonuclease-mediated incorporation (REMI), with the improvement of the addition of inverted terminal repeat sequences (ITRs) from adeno-associated virus (AAV) to the plasmid. ITRs enhance transgene expression in mammal cells. CS2nGal was digested with Sall and SStII, filled in, and ligated to a filled-in XbaI fragment from plasmid p2L, containing two copies of the right AAV ITR. The DNA was linearized prior to microinjection. Inclusion of ITRs on plasmid DNAs increased the ability of both linearized and closed circular DNAs to segregate more efficiently throughout the embryo, increasing tissue-specific expression as well as efficiency of expression. The new strategy may have applications to other vertebrate systems. (19 ref)

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0219967 DBR Accession No.: 98-01564

Tissue-specific expression of herpes simplex virus thymidine-kinase gene delivered by adeno-associated virus inhibits the growth of human hepatocellular carcinoma in athymic mice - gene transfer and ganciclovir prodrug activation for cancer gene therapy

AUTHOR: Su H; Lu R; Chang J C; Kan Y W

CORPORATE AFFILIATE: Howard-Hughes-Med.Inst. Univ.California  
CORPORATE SOURCE: Department of Laboratory Medicine, University of California, Third and Parnassus Avenues, Room U426, San Francisco, CA 94143-0724, USA.

JOURNAL: Proc.Natl.Acad.Sci.U.S.A. (94, 25, 13891-96) 1997  
ISSN: 0027-8424 CODEN: PNASA6

LANGUAGE: English

**ABSTRACT:** A recombinant adeno-associated virus vector was constructed for

herpes simplex virus thymidine-kinase (EC-2.7.1.21) gene transfer to human hepatocellular carcinoma cell line PLC/PRF/5. The suicide gene was under the control of an alpha-fetoprotein enhancer and albumin promoter. S.c. tumors generated in nude mice by implanting the transduced hepatocellular carcinoma cells shrank dramatically after treatment with ganciclovir. A bystander effect was also observed on the tumors generated by mixing transduced and untransduced cells. Thus, suicide gene transfer and prodrug activation may be used for cancer gene therapy. (25 ref)

10/7/35

DIALOG(R)File 357:Derwent Biotech Res.

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0219885 DBR Accession No.: 98-01482

Gene transfer and expression in oligodendrocytes under the control of myelin basic protein transcriptional control region mediated by adeno-associated virus - for neurological disease gene therapy

AUTHOR: Chen H; McCarty D M; Bruce A T; Suzuki K; +Suzuki K

CORPORATE AFFILIATE: Univ.North-Carolina

Brinkhouse-Bullitt Building, University of North Carolina, Chapel Hill,  
NC 27599-7525, USA.

JOURNAL: Gene Ther. (5, 1, 50-58) 1998

ISSN: 0969-7128 CODEN: 4352W

LANGUAGE: English

**ABSTRACT:** An adeno-associated virus (AAV) vector carrying a humanized green fluorescent protein (GFP) reporter gene, linked to the transcriptional control region from a myelin basic protein (MBP) gene (a myelin-forming cell-specific gene) was constructed. Oligodendrocyte transduction was carried out in vitro and in vivo. GFP expression was detected for at least 3 wk in both a transduced oligodendrocyte cell line (MOCH-1 cells) and rat oligodendrocyte primary cultures. Preferential GFP expression in oligodendrocytes occurred in primary cultures. In contrast, transduction with an AAV vector carrying a cytomegalovirus promoter produced stronger GFP fluorescence in various cell types, with the strongest signal from astrocytes. Infusion of 6,000 million particles (200,000 infectious units) of rAAV-MBP-GFP into mouse brains resulted in GFP expression specifically in white matter. GFP protein was detected 15 days later in oligodendrocytes, and no astrocytes were transduced. The MBP transcriptional control region may be useful in neurological disease gene therapy strategies targeted to myelinating cells. (28 ref)

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DIALOG(R)File 357:Derwent Biotech Res.

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0215702 DBR Accession No.: 97-10823 PATENT

Delivering gene to muscle cell or tissue using recombinant adeno-associated virion - for use in gene therapy and as recombinant vaccine

AUTHOR: Podskoff G M; Kessler P D; Byrne B J; Kurtzman G J

CORPORATE SOURCE: Alameda, CA, USA; Baltimore, MD, USA.

PATENT ASSIGNEE: Avigen; Univ.Johns-Hopkins 1997  
PATENT NUMBER: WO 9726337 PATENT DATE: 970724 WPI ACCESION NO.: 97-385340 (9735)

PRIORITY APPLIC. NO.: US 784757 APPLIC. DATE: 970116  
NATIONAL APPLIC. NO.: WO 97US895 APPLIC. DATE: 970117

LANGUAGE: English

**ABSTRACT:** A new composition useful for delivering a selected gene to a muscle cell (preferably a skeletal myoblast, skeletal myocyte or cardiomyocyte) or tissue (e.g. derived from skeletal, smooth or cardiac muscle) contains a recombinant adeno-associated virus (AAV) virion containing the target gene linked to control elements. The gene preferably encodes a therapeutic protein, especially acid alpha-glucosidase (EC-3.2.1.20). The control elements consist of an

inducible muscle-specific promoter sequence. Also claimed is a muscle cell or tissue transduced in vitro with the recombinant AAV virion. The virions may be used for treating type II glycogen storage disease. The target gene may also encode erythropoietin and other proteins capable of treating endocrine, metabolic, hematological and cardiovascular diseases including AIDS, cancer and diabetes. The virions are non-pathogenic and may be used for the delivery of antigens for immunization. Cells transduced provide sustained, high-level expression of the target gene, and the protein is secreted to provide systemic delivery. (76pp)

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DIALOG(R)File 357:Derwent Biotech Res.

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0209380 DBR Accession No.: 97-04501 PATENT

System for expressing heterologous protein or gene exclusively in mature T-cells - human CD4 or herpes simplex virus-1 thymidine-kinase gene transfer to hematopoietic stem cell for gene therapy; vector with a human or mouse CD4 gene amplifier region

AUTHOR: Klattmann D; Salmon P; Boyer O

CORPORATE SOURCE: Paris, France.

PATENT ASSIGNEE: Univ.Paris-Pierre-Marie-Curie 1997  
PATENT NUMBER: WO 9704118 PATENT DATE: 970206 WPI ACCESION NO.: 97-132655 (9712)

PRIORITY APPLIC. NO.: FR 958616 APPLIC. DATE: 950717  
NATIONAL APPLIC. NO.: WO 96FR1122 APPLIC. DATE: 960717

LANGUAGE: French

**ABSTRACT:** A new expression system uses a vector for transduction of hematopoietic stem cells, so that the transgene is only expressed in mature T-lymphocytes after differentiation. The vector includes

expression sequences (e.g. a human CD4 promoter, a polyadenylation signal, and a human CD8 gene silencer), and has at least 1 amplifier from a human or mouse CD4 gene. A human CD4 cDNA or a herpes simplex virus-1 thymidine-kinase (EC-2.7.1.21) suicide gene for aciclovir or ganciclovir prodrug activation may also be included. The vector may be a retro virus, adeno virus, adeno-associated virus or non-biological DNA delivery system. The system may be used in gene therapy for selective destruction of activated T-lymphocytes, particularly to prevent graft rejection or graft-versus-host disease, or in therapy or prevention of HIV virus infection, autoimmune disease or primary or secondary immunodeficiency. The vector confers specific expression in mature but not immature T-lymphocytes. The need to transfect mature cells in vitro is avoided, and there is no danger that suicide genes will kill permanently dividing cells. (51pp)

10/7/45  
DIALOG(R)File 357:Derwent Biotech Res.

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0208076 DBR Accession No.: 97-03197

Efficient transduction of green fluorescent protein in spinal cord neurons using adeno-associated virus vectors containing cell type-specific

promoters - neuron-specific enolase and platelet-derived growth factor

B-chain promoter-containing vector-mediated humanized gene transfer to

rat neuron for central nervous system gene therapy

AUTHOR: Peel A L; Zolotukhin S; Schrimsher G W; Muzychka N; Reier P J

CORPORATE SOURCE: Department of Neuroscience, University of Florida Brain Institute, Box 100244, Gainesville, FL 32607, USA.

JOURNAL: Gene Ther. (4, 1, 16-24) 1997

ISSN: 0969-7128 CODEN: 4352W

LANGUAGE: English

**ABSTRACT:** The ability of recombinant adeno-associated virus (rAAV) vectors, containing cell type-specific promoters, to transduce (*in vivo*) normal

adult Sprague-Dawley rat spinal cord neurons was investigated for central nervous system disease gene therapy. A neuron-specific enolase

(EC-4.2.1.11) (NSE) promoter and a platelet-derived growth factor

B-chain (PDGF) promoter were used to direct humanized green fluorescent protein (GFP) gene expression. Neuron-specific rAAVs were injected into the mid-cervical regions of adult rat spinal cords. At 10-14 days, expression was detected in all animals and persisted for up to 15 wk.

Immunocytochemical and morphological profiles of transduced cells were consistently neuronal, and there was no evidence of transgene expression in glial elements. Transduction efficiencies for the NSE and PDGF rAAVs were estimated at 15 and 45 infectious particles per GFP-positive neuron, respectively, in the absence of detectable adeno virus. Thus, rAAVs may be used to enhance spinal cord repair following injury. (39 ref)

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DIALOG(R)File 357:Derwent Biotech Res.

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0194968 DBR Accession No.: 96-05739

**LANGUAGE:** English

**ABSTRACT:** Recombinant adeno-associated virus (AAV) vectors, containing the neomycin-resistance gene under the control of the herpes thymidine-kinase promoter and a normal human beta-globin gene marked with a Clal linker ( $\nu$ -beta-m-globin), or the DNA-asc-I (EC-3.1.21.1) hypersensitivity site-2 (HS2) from the locus control region of the human beta-globin gene cluster ( $\nu$ -HS2-beta-m-globin), were constructed. These vectors were subsequently used to transfect the human erythroleukemia K562 cell line, or the human nasopharyngeal carcinoma KB cell line and cell populations resistant to G418 were obtained. There was no expression if the beta-globin gene in K562 or KB cells infected with the v-beta-m-globin virus. However, high level transgene expression was demonstrated in 10-20% of  $\nu$ -HS2-beta-m-globin virus-infected K562 cells. These results suggest that the transduced human beta-globin gene was expressed in an erythroid cell-specific manner and that the AAV-based vector system may prove useful for globin gene transfer in human hematopoietic stem cells. (54 ref)

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DIALOG(R)File 357:Derwent Biotech Res.

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0192561 DBR Accession No.: 96-02754

Evaluation of adeno, adeno-associated and herpes simplex viral vectors for *in vivo* gene delivery to the mouse retina - herpes simplex virus, adeno virus and adeno-associated virus vector and promoter comparison for potential use in retinal degeneration gene therapy (conference abstract)

AUTHOR: Ali R R; Reichel M B; Kanuga N; Thrasher A; Byrnes A; Hunt D M ; Bhattacharya S S

CORPORATE SOURCE: Department of Molecular Genetics, Institute of Ophthalmology, University College, London EC1V 9EL, UK.

JOURNAL: Gene Ther. (2, Suppl.1, S3) 1995

ISSN: 0969-7128 CODEN: 4352W

CONFERENCE PROCEEDINGS: Human Gene Transfer and Therapy, 3rd Meeting, Sitges, Barcelona, Spain, 17-20 November, 1995.

LANGUAGE: English

**ABSTRACT:** Vectors were compared for potential use in gene therapy of inherited retinal degenerations. The ability of adeno virus type 5 (AV), adeno-associated virus (AAV) and herpes simplex virus type 1 (HSV) to transduce retinal cells *in vivo* with a reporter gene was studied using subretinal or intravitreal injection in neonatal and adult mice. Subretinal injections of AV carrying a Rous-sarcoma virus (RSV)-driven lacZ gene resulted in gene expression in the retinal pigment epithelium with less in photoreceptor cells. This decreased over 3 wk. AV carrying a cytomegalovirus-driven lacZ reporter transduced more photoreceptors than AV with the RSV promoter. Whilst AV

JOURNAL: Gene Ther. (3, 3, 223-29) 1996

ISSN: 0969-7128 CODEN: 4352W

was easily produced, it was immunogenic and too large to penetrate the retina on intravitreal injection. AAV were studied using a CMV-driven lacZ reporter. The size of this virus may allow the target cells to be reached by intravitreal and subretinal injection. The difficulty of AAV production and its smaller packaging size limited its usefulness. HSV was much larger and carried larger genes. It was efficient at infecting photoreceptor cells, but caused cell death. (0 ref)

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0192559 DBR Accession No.: 96-02752

Adeno-associated virus delivery of an opsin promoter driven reporter gene to the mouse and rabbit retina - vector construction with opsin promoter and in vitro packaging for beta-galactosidase eye tissue-specific gene expression; potential gene therapy (conference abstract)

AUTHOR: Hauswirth W W; Zolotukhin S; Muzychka N; Flannery J G

CORPORATE AFFILIATE: Univ Florida Univ.California

CORPORATE SOURCE: Department of Ophthalmology, University of Florida

College of Medicine, Gainesville, FL 32611, USA.

JOURNAL: Gene Ther. (2, Suppl.1, S2) 1995

ISSN: 0969-7128 CODEN: 4352W

CONFERENCE PROCEEDINGS: Human Gene Transfer and Therapy, 3rd Meeting,

Sitges, Barcelona, Spain, 17-20 November, 1995.

LANGUAGE: English

**ABSTRACT:** The human adeno-associated virus-2 (AAV-2) was used for gene transfer to mouse and rabbit retina following intraocular injection. A 400 bp fragment of the 5' flanking region of the mouse opsin gene was used to direct specific expression of beta-galactosidase (B-Gal, EC-3.2.1.23), a marker for the cellular site of expression in the eye. B-Gal expression was traced by Lac-Z staining and reverse transcription polymerase chain reaction. Rabbits and mice received intravitreal and sub-retinal injections of AAV. High level gene expression occurred in the neural retina (photoreceptors and retinal neurons) of all eyes studied. No eye inflammation or pathology was observed. The regional expanse of B-Gal activity correlated with the volume of viral suspension injected and the viral titer. An in vitro reaction for packaging recombinant AAV DNA was developed. Using AAV replicative form DNA as the substrate, an infectious AAV virus was synthesized that could be used to transfer a B-Gal gene to mammalian cells. The packaging reaction required both the AAV Rep and capsid proteins and will aid AAV virus production for gene therapy. (0 ref)

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DIALOG(R)File 155: MEDLINE(R)

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10870117 97221745 PMID: 9068791

Efficient transduction of green fluorescent protein in spinal cord neurons using adeno-associated virus vectors containing cell type-specific promoters.

Peel A L; Zolotukhin S; Schirmer G W; Muzychka N; Reier P J  
Department of Neuroscience, University of Florida Brain Institute,  
Gainesville 32607, USA.

Gene therapy (ENGLAND) Jan 1997, 4 (1) p16-24, ISSN 0969-7128  
Journal Code 9421525

Contract/Grant No.: GM 3572302; GM, NIGMS, HL/DK 50257; HL; NHLBI; MH 15737; MH; NIMH

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In this study, we have evaluated the capacity of recombinant adeno-associated virus (rAAV) vectors, containing cell type-specific promoters, to transduce neurons *in vivo* in the normal adult rat spinal cord. The neuron-specific enolase (NSE) promoter and the platelet-derived

growth factor (PDGF) B-chain promoter were used to direct expression of a 'humanized' form of the gene for green fluorescent protein (GFP). Neuron-specific rAAVs were injected into the mid-cervical regions of adult rat spinal cords. At 10-14 days, expression was detected in all animals and persisted for up to 15 weeks. Immunocytochemical and morphological profiles of transduced cells were consistently neuronal, and there was no evidence of transgene expression in glial elements. Transduction efficiencies for the NSE and PDGF rAAVs were estimated at 15 and 45 infectious particles per GFP-positive neuron, respectively, in the absence of detectable adenovirus. This study strongly supports a role for rAAV vectors in CNS gene therapy and lays the groundwork for delivery of more functional genes to spinal cord neurons as a possible way to enhance spinal cord repair following injury.

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